Nematode DNA Isolation using Qiagen DNAEasy Kit (cat #69506)

by Robyn Tanny July 2014

1. Chunk desired strain of nematode to two 10 cm HGMA (High Growth Medium) plates (recipe below).

2. When the 10 cm plates are just about starved, wash the nematodes off the plates:
   a. Squirt M9 on one plate, transferring the liquid to the other plate.
   b. Pour (or pipette using a Pasteur pipette) the worms into a labeled 15 ml conical tube.
   c. Repeat steps a and b.

   NOTE: If the plates are too starved (ie - the worms have started to burrow), you can increase your yield by washing the worms off into the conical, allow the plate to sit for 5-10 minutes, wash the plate again into a second 15 ml conical. Waiting after some liquid is on the plate might help flood the burrowed worms back to the surface.

3. Allow the worms to settle in the conical. This will take about one hour.

4. Aspirate off the M9.

5. Add 5 ml of M9 to the 15 ml conical and allow the worms to settle again.

6. Aspirate off the M9. Transfer the worms in the remaining liquid to a labeled 1.5 ml minicentrifuge tube. Add a little (less than 1 ml) M9 to the conical and transfer any remaining worms to the microcentrifuge tube.

7. Allow the worms to settle.

8. Aspirate off the majority of the M9 and use a pipetteman to manually remove the remaining liquid. If you aspirate off all the liquid, you are also removing worms.

9. Optional: At this point, the worms can be stored at -80°C. This *might* help with cracking the cuticule, but it doesn't seem to make much difference. However, the worms can be stored at -80°C for several weeks until you are ready to isolate DNA.

10. Turn on a heat block to 56°C. Add 180 ul of Buffer ATL and 20 ul of Proteinase K (provided with the kit). Incubate at 56°C with vortexing (or vortex a few times during incubation). Check the amount of lysis after 1 hr. If you still see worms, continue the incubation. If you see embryos or nothing, proceed to the next step.

11. Add 4 ul of RNAseA (100 mg/ml) (not provided with kit; use your favorite RNAseA). Incubate at room temperature for two minutes.

12. Add 200 ul buffer AL. Incubate at 56°C with vortexing (or vortex a few times during incubation) for 10 minutes.

13. Add 200 ul EtOH and vortex to mix. Transfer contents to a labeled spin column in a collection tube.

14. Spin at 10000 rpm for one minute. NOTE: if all the contents did not go through the column, repeat the spin.

15. Remove spin column and transfer to a new collection tube. Add 500 ul Buffer AW1.

16. Spin at 10000 rpm for one minute.

17. Remove spin column and transfer to a new collection tube. Add 500 ul Buffer AW2.

18. Spin at maximum speed (13000-14000 rpm) for three minutes.

19. Remove spin column and transfer to a clean, labeled 1.5 ml microcentrifuge tube. Add 200 ul Buffer AE.

20. Incubate at room temperature for one minute.

21. Spin at 10000 rpm for one minute.

22. Remove spin column and transfer to a clean, labeled 1.5 ml microcentrifuge tube. Add 200 ul Buffer AE.

23. Incubate at room temperature for one minute.

24. Spin at 10000 rpm for one minute.

25. Combine eluates.

26. Determine DNA concentration using Qubit Broad Range (cat# Q32850; follow Qubit-provided protocol).

27. If the concentration is very low, you can concentrate the sample using a speed-vac.

HGMA2 Recipe

	1 L	**2 L**
Peptone	20 g	40 g
NaCl	3 g	6 g
Agarose	20 g	40 g
Sterile water	975 mL	1950 mL